

A Simple Continuous-Flow Toxicant
Delivery System

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A SIMPLE CONTINUOUS-FLOW TOXICANT DELIVERY SYSTEM

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Abstract—This paper describes a simple continuous-flow toxicant delivery system for use in testing toxicity of dissolved or suspended compounds to aquatic organisms. Its advantages are simplicity, ease of construction, and capacity for high flow rates. The system consists of a glass headbox and diluter box, a toxicant pump, a framework for support, and a system of tubes to deliver a series of toxicant concentrations to the test aquaria. Methods are given for construction and for calibration of the system.

INTRODUCTION

Toxicity testing with aquatic organisms requires tanks or other facilities for holding the test organisms and a reliable, accurate method of delivering the toxicant to the test subjects. Reliable toxicant delivery systems have been available for some time, two popular examples of which are described by Mount & Warner (1965) and Mount & Brungs (1967). The advantages of the system here described are its simplicity, its ease of construction and maintenance, and its capacity to be modified for a wide variety of flow rates and concentrations. In the latter respect the system described is unique because it can deliver much greater flows than other systems described in the literature. For example, Mount & Brungs (1967) state that their diluter can provide flows up to 400 ml/min whereas the system described here can provide flows ranging from less than 400 ml/min to several l/min. A possible disadvantage is that very low flows require small tubing which may become clogged by solids or by growths of algae or bacteria. This potential problem can be largely eliminated by proper filtration or screening and by proper light shielding and cleaning of the tubes.

This system uses the principle of serial dilution described by Warner (1962) whereby one source of toxicant is diluted by successive additions of water to produce lower and lower concentrations of toxicant. Mount & Warner (1965) stated that, "serial dilution has many advantages: it is more accurate in most cases, fewer controlling valves are needed, only one stock solution is required, and there are fewer small constrictions to be clogged".

The diluter's reliability has been demonstrated in more than 5 years' operation at Western Fish Toxicology Station. During this time the only serious malfunctions have been due either to loss of water pressure to the system or to loss of electrical power to the toxicant pump. In tests conducted with dissolved cadmium at a concentration of 3.0 µg/l, copper at 30 µg/l and zinc at 700 µg/l variability ranged from a maximum of 12% with copper to a minimum of 2% with

zinc over a period of weeks. This, of course, includes variability inherent in the chemical analyses. WFTS data from similar tests using the Mount & Brungs (1967) diluter show that the two systems are not significantly different in their variability.

The complete toxicant delivery and testing system in use is 2.13 m long by 2.4 m high by 1.2 m wide, including a stand for the dilution apparatus and twelve test tanks. However, the dilution apparatus alone could fit into a space 61 cm long by 30.5 cm wide by 61 cm high if a different test tank arrangement is desired. The diluter delivers 21 l. of toxicant per hour at each of five concentrations, plus a control. With larger tubes it can deliver several times that amount, and the number of concentrations can be changed to best suit the test.

PRINCIPLES OF OPERATION

The unit (Fig. 1) consists of: (1) clean water source (the headbox); (2) toxicant source (constant rate drip system); (3) mixing chambers (the diluter box); (4) delivery system to the test tanks; and (5) support sys-

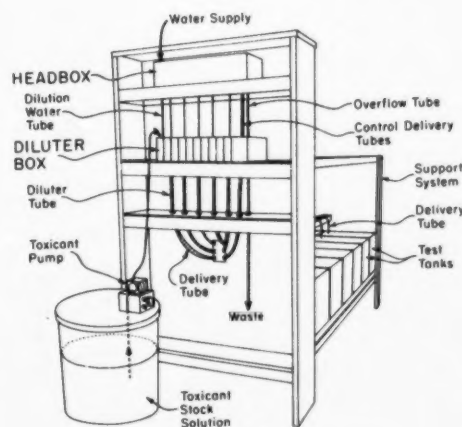


Fig. 1. Overall view of test system.

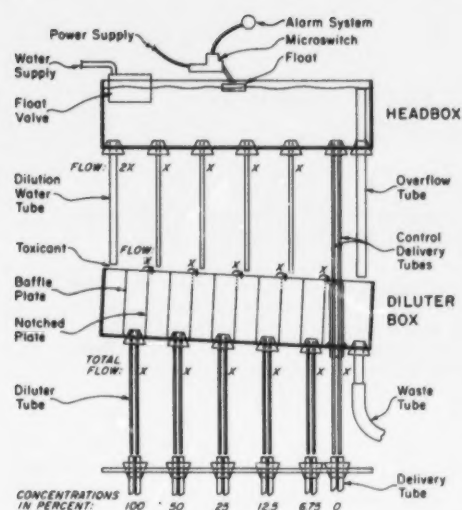


Fig. 2. Headbox, diluter box and delivery tubes set up for 50% dilution.

tem for dilution apparatus and test tanks. In the dilution apparatus toxicant is added to the first chamber of the multi-chambered diluter box (Figs. 2 and 3). This toxicant is mixed with fresh water from the headbox to produce the highest concentration of toxicant desired. This concentration can be adjusted by changing the strength of stock solution, the amount of stock solution delivered, or the amount of clean dilution water added.

Part of the solution from the first chamber is routed to the test tanks but, by design, the toxicant and dilution water added to the chamber have more total flow than the diluter tubes will accept. The excess spills through a notch into the next dilution chamber and becomes the toxicant source for this chamber. The same dilution and overflow occur in this and each succeeding chamber. A last small amount of dilute toxicant is wasted in the overflow at the end of the diluter box.

Flow to the test tanks depends upon size of delivery tubes and hydraulic head for the tubes. Dilution factor depends upon the relationship between dilution water flow into a chamber and overflow from preceding chamber (amount of toxicant supplied). These are regulated by tube size.

MATERIALS

Headbox and diluter box are both made of 30.5-cm by 61-cm double-strength glass sheets or sections cut from such sheets. The size was chosen to make use of commercial size glass cut in multiples of 1 ft. The sheets are joined together by translucent silicone rubber adhesive sealant, and a bead of the adhesive sealant is added and smoothed in by fingertip to the inside corners later to prevent leakage. Tubes are of standard size glass tubing cut to 29.8 cm so that four tubes can be cut from a 122 cm section with allowance for cuts and squaring of the ends. Standard size neoprene stoppers are used to join tubes to the diluter and headbox. A float valve is used to ensure a constant level in the headbox. Simple float valves designed for stock-watering tanks have been quite satisfactory, but such valves must not have water contact surfaces of zinc or other toxic substances. A metering pump was chosen to deliver toxicant to the diluter box because it has a fine adjustment capability and a wide-range pumping capacity, and because it is accurate. The toxicant also may be added by means of a drip tube from a constant-head toxicant box. In this case, stock concentration is adjusted rather than rate of flow. The optional alarm system described requires a microswitch and float, light fixtures, buzzer or other alarm, and a small amount of electrical wiring.

The dilution apparatus alone may be made for under \$50 in the size described here, including a \$10 stock-watering float valve for the headbox. A simple alarm system using a microswitch and light can be made for less than \$20. A support system like the one shown for the test tanks will cost about \$80 if made of plywood and slotted angle iron and less if made en-

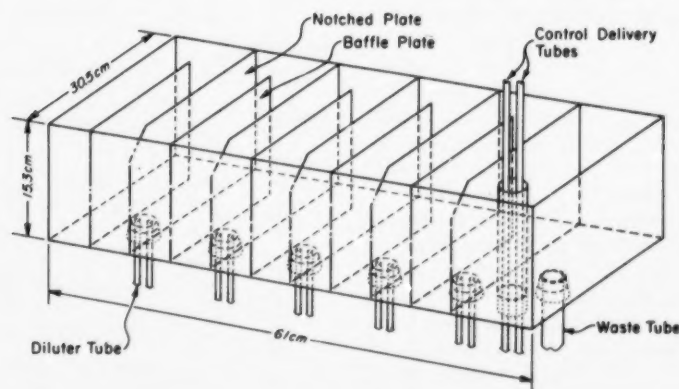


Fig. 3. Detail of diluter box.

tirely of wood. The toxicant supply source is the major variable in price and this could range from under \$50 for a general purpose pump supplying a constant-head box with drip tube to the diluter to over \$300 for a precisely-controlled pump which delivers directly to the diluter.

CONSTRUCTION

Support system

The support for the test tanks consists of a frame 1.2 m wide by 2.13 m long built like a "bunk bed" to provide a lower platform for twelve 38-l. aquaria and an upper platform for storage and for support of lights or plastic screening to isolate the test tanks. The diluter frame, which forms the "headboard" of the "bed" is 2.4 m high by 1.2 m wide by 0.3 m deep. It must be high enough to provide gravity flow from headbox to diluter box to test tanks through the delivery tubes, but, if this provision is met, the dimensions can be altered to fit each specific situation. The support system described was constructed of slotted angle steel framework and 1.9 cm plywood shelves. Holes are cut for tubes to pass through, and shelves are then painted with waterproof enamel.

Headbox

The headbox is made from three pieces of 30.5-cm by 61-cm double-strength glass. The bottom 30.5-cm by 61-cm sheet is drilled as in the diluter box (Fig. 2) with a 2.54-cm glass drill to accommodate seven No. 3 neoprene stoppers which hold the overflow tube and tubes to the diluter box. Later versions of the diluter box have been built with stainless steel bottoms. This adds strength but increases the cost. The long sides are 15.3 cm by 61 cm, and the ends are 15.3 cm by 29.9 cm. By cutting the ends 29.9 cm the side thickness is accounted for and the two ends butt against the sides to form a rectangle that exactly fits over the 30.5-cm by 61-cm bottom. Sides and ends are properly placed and secured to the bottom with clear silicone cement.

Diluter box

The diluter box is made to the same dimensions as the headbox, and holes are drilled as shown in Figs 2 and 3. Five baffle plates 15.3 cm $\times$ 25 cm are inserted which join to the front wall but leave a space between them and the back wall to provide for further travel and more opportunity for mixing before the toxicant goes to the delivery system.

The diluter box is divided into six different compartments in the example diluter although more, or fewer, compartments are possible. The compartments are divided by 15.3-cm by 29.9-cm glass plates, each of which has a top corner cut off where the divider plate meets the front side plate of the diluter. Divider plates are glued in with silicone sealant, and a bead is smoothed into the joints with a fingertip to prevent leakage from one chamber to the next. The overflow

notch on the example diluter was made by cutting a 45° triangle, 2.54 cm on a side, off the corner of the divider sheet. If the diluter is made for higher flows, the notch may have to be cut larger to carry the overflow from one compartment to the next. In this case one may wish to make the diluter box sides 30.5 cm high instead of 15.3 cm to reduce splashing.

Toxicant metering system

The toxicant metering system is not necessarily a part of the diluter apparatus but may be designed to fit the degree of accuracy and precision required and to conform to budgetary limitations. This system delivers the toxicant from a stock solution in a bottle or tank, depending upon the amount to be used, to the first chamber of the diluter box. The concentration delivered from the first diluter chamber is determined by the concentration and rate of flow of toxicant put into the chamber. All subsequent concentrations are dilutions of the first. Within the limits of solubility, the concentration can be controlled when the stock solution is made up. Flow of toxicant is determined by the metering system.

Perhaps the easiest method is by precise metering pump between stock solution and diluter box. This allows for easy adjustment of flow to match stock solution and concentration desired in the diluter box but it is the most expensive method. Another satisfactory way has been to pump into a small chamber with an overflow placed higher than the diluter box. The overflow maintains a constant head in the chamber and toxicant is delivered at a constant flow rate through a restricted glass tube to the diluter box. This method is less expensive since a simple, less precise pump can be used but new drip tubes must be made each time flow rate is changed. In this system the amount of toxicant delivered is controlled by adjustment of stock solution concentration.

A third method of metering is by using a Mariotte bottle as a stock solution source. This bottle is used to maintain a constant head in an adjacent box which supplies toxicant through a restricted glass tube directly to the diluter box. Flow is determined by restriction of the tube and concentration is adjusted in the stock solution. This system can be less expensive than the first two but it limits the amount of stock solution since all must be carried in the Mariotte bottle. This would be a disadvantage in long-term experiments.

Delivery system

The delivery system from the diluter box to the test tanks should be nontoxic and should not leach or adsorb materials that will alter the character of the solution being tested. Glass tubing works very well with most solutions. Connections are made with polyvinyl chloride (PVC) or other nonreactive tubing used over the glass to hold the glass tubes butted together with the least exposure of non-glass material. In a very large system pipe can be used instead of glass

tubing if the pipe is made of glass or other material known to be nontoxic and nonreactive with the materials to be tested.

Since this is a gravity flow system, delivery tubes should be of larger diameter than the diluter tubes to ensure adequate delivery capacity and a free flow. Diluter tubes fit inside the larger delivery tubes and there must be an air break between delivery tubes and the diluter tubes to ensure that there is no suction on the diluter tubes. Otherwise, the hydraulic head on the diluter tubes would vary with length of the delivery system and test tanks would not be assured uniform flows.

Alarm system

An alarm system is not a necessary part of the apparatus, but it is highly recommended and can easily pay for itself in preventing lost experiments. The system used at WFTS and described below provided a red indicator light, automatic shutoff of toxicant supply, and tie-in to a commercial answering service.

The sensor unit (Fig. 2) is a microswitch activated by a lever arm attached to a 250-ml polyethylene bottle which serves as a float on the surface of the headbox. The switch is in the open position when the float is at normal water level. If the water level drops, the switch closes. This sends current to a double-pole, double-throw relay switch that cuts off the toxicant pump and closes a contact wired into the alarm system.

Calculation of dilution factor

Dilution factor is determined by the ratio of toxicant solution to dilution water for each chamber. The amount of toxicant in the first chamber is controlled by the pump or other device metering the initial supply. The amount of toxicant in each succeeding chamber depends upon the concentration and volume of overflow from the preceding chamber. Dilution water is controlled by varying the diameter or hydraulic head, or both, of the water-supply tubes coming from the headbox.

A setup for 50% dilution is shown in Fig. 2. The first chamber has a total incoming flow of $2x$, plus the

Table 1. Flows obtained with Kimax* glass tubes with tube ends ground off square (tube length = 27 cm; water depth over tube entrance = 13 cm; head = 40 cm)

Tube diameter (mm)	Flow (l/min)
4	0.35
6	1.06
8	2.53
10	4.79
12	8.18
16	16.73

* Mention of commercial products does not constitute endorsement by the U.S. Environmental Protection Agency.

Table 2. Flow changes in 8 mm diameter tubing with changes in head

Water depth (cm)	Tube length (cm)	Head (cm)	Flow (l/min)
12.5	5	17.5	2.09
12.5	10	22.5	2.19
12.5	15	27.5	2.31
12.5	30	42.5	2.47

small amount of added toxicant. Each subsequent chamber also has a total incoming flow of $2x$, equal amounts of toxicant and dilution water. Then, x amount goes to the test tanks and the remaining x amount goes to the next chamber or, at the last chamber, to waste. If the incoming flow is changed to x amount of toxicant and $2x$ amount of dilution water, the concentration in each chamber will be 33% of that in the preceding chamber. If toxicant flow is x and dilution flow is $0.5x$, each chamber will have a concentration of 66% that in the preceding chamber. The amount of toxicant passed between chambers is equal to the toxicant flow into the chamber, plus the dilution water flow into the chamber, minus the flow out of the chamber to the test tanks.

Flow rates are regulated by choice of tube diameter and hydraulic head. Equations to calculate the flow of different tube sizes requires a knowledge of head loss per unit of tubing caused by friction or entrance condition. Since the constants for the equations are difficult to obtain, the author has found it more practical to test actual lengths of tubing to determine which sizes give the required flow. Flows for some representative sizes are shown in Table 1.

Hydraulic head can also be altered to make small flow adjustments after assembly, although this generally is not necessary. One must keep in mind that head is determined by total distance from the water surface to the bottom end of the tube. Table 2 shows how head changes can change flow through 8 mm diameter tubing with square-cut ends.

After tubes have been chosen and diluter is assembled, dilution factor should not change if tubes are kept clean. Cleaning can be accomplished by a combination of acid wash and mechanical scrubbing with a swab or pipe cleaner. To change dilution factor or rate of flow to test tanks, simply change to different size tubing in the supply and delivery systems.

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